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Basic HPLC Troubleshooting

Technical Note CS06

The most common problems and solutions observed in HPLC separations are outlined in this technical note.

Basic troubleshooting involves systematically isolating the problem and seeing if it is reproducible. Once the problem is isolated, the next step is to change only one variable at a time to see if it resolves the problem. Always document the problem and solution for future reference, to reduce the amount of time spent troubleshooting.

Problem: Extra or ghost peaks

Probable Causes	*Solution*
Peaks from previous injection	Extend analysis run time or flush column with stronger eluent at end of run time.
Column contamination	Improve sample clean-up by adding either a filtration or solid phase extraction step to sample preparation prior to analysis. High back pressure can be an indication of contaminant buildup at the head of the column. Disconnect the column from the detector reverse the direction of flow through the column and backflush. It maybe necessary to remove strongly retained contaminants with a series of miscible solvents. An example of a solvent rinse for heavily contaminated reverse phase columns is water, acetonitrile, tetrahydrofuran, methylene chloride, hexane and reverse the order until back to water. Refer to the column manufacturer's instructions for rinsing heavily contaminated columns.
Contaminated solvents or water	Use HPLC grades solvents and water for mobile phase preparation. Filter all mobile phases through a 0.45μm or 0.20μm prior to use. Use solvent reservoir and in-line filters to trap particulate. Store mobile phase in glass containers to avoid contamination from plasticizers.

Problem: Tailing Peaks

Probable Causes	*Solution*
Basic analytes interacting with surface silanols	For reverse phase columns, use an endcapped column that has been treated to reduce free surface silanol groups. Triethylamine (TEA) can be added to MP to irreversibly react with free surface silanols.
Metals in silica	Use high purity silica based columns with low metal content.

Problem: Broad Peaks

Probable Causes	*Solution*
Sample overload	Reduce injection volume or sample concentration.
Extra column volume	Small area peaks that are quite broad often indicate excessive extra column volume between the injector and the detector. Use zero dead volume connections and the minimum tubing diameter/length required.
Too long a run time	If possible use gradient elution program or stronger mobile phase.
Poor column efficiency	Use a column with a smaller particle size to increase column efficiency.
Blocked column frit	Disconnect column from detector, reverse flow and rinse to remove blockage.

Problem: Split Peaks

Probable Causes	*Solution*
Sample volume too large	Reduce injection volume or sample concentration.
Injection solvent too strong	The sample should be diluted in mobile phase when possible to avoid peak shape distortion when the sample diluent is stronger than the mobile phase.
Column void or channeling	Replace column.
Blocked column frit	Reverse column flow and rinse column. Installation of 0.2-0.5μm in-line filter after the injector can reduce the amount of particulate contaminating the analytical column.
Change in mobile phase composition	If only one or two peaks are splitting, resolution may have been reduced due to changes in mobile phase composition. Prepare fresh mobile phase.
Contamination	Additional sample clean up with filtration or solid phase extraction may be required to remove interfering peak.
Channels present in column	Replace analytical column.

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Problem: Changing Retention Times

Probable Causes	*Solution*
Fluctuations in column temperature	Use a column heater or chiller to maintain temperature stability
Insufficient column equilibration during gradient run	Increase equilibration time between injections. Allow at least 10-15 column volumes of mobile phase to pass through the column prior to sample injection.
Contamination build-up	Improve sample clean-up by adding either a filtration or solid phase extraction step to sample preparation prior to analysis. High back pressure can be an indication of contaminant buildup at the head of the column. Disconnect the column from the detector reverse the direction of flow through the column and backflush.
Changes in mobile phase composition	Keep solvent reservoirs covered to prevent evaporation. Buffered mobile phases should be prepared fresh. Helium sparging could increase evaporation of volatile solvents. Use of in-line solvent degasser can reduce evaporation caused by helium sparging.
Column aging	Column life can be extended with the use of a guard column. Replace analytical column.

Problem: Baseline Noise

Probable Causes	*Solution*
Contamination	Random base line noise or baseline drift often indicates contamination. Flush column especially after using buffered mobile phases, backflush column if necessary. Improve sample clean-up prior to injection by filtering sample through 0.45μm or 0.20μm syringe filter or adding a solid phase extraction step. Use HPLC grade solvents, reservoir filters and inline filters to trap contaminants.
Detector lamp deterioration	Continuous baseline noise often indicates lamp deterioration, replace lamp. Most UV detector lamps last approximately 1000 hours.
Air bubbles	If the baseline noise stops when the flow is turned off, there is likely an air bubble in the detector. A back-pressure regulator on the outlet of the flow cell can help remove air bubbles. Oscillating back pressure is frequently caused by inadequate degassing of the mobile phase(s).
Electrical interference	Occasional baseline noise could be due to electrical interference by water baths and other instrumentation. Use of a voltage stabilizer can reduce noise due to electrical interference

Problem: High Column Back Pressure

Probable Causes	*Solution*
Column blockage or blocked frit	Reverse solvent flow and backflush column while disconnected from detector.
Particle size too small	Use a column with a larger particle size.
Mobile phase too viscous	Use lower viscosity solvents or increase column temperature.
Microbial growth in mobile phase	Prepare aqueous buffers fresh daily, or add >10% organic solvent to prevent microbial growth.

Problem: Drop in system pressure or leaks

Probable Causes	*Solution*
Loose fitting	If there is precipitate around the fittings, re-tighten fitting or replace ferrule and tubing if necessary.
Pump seal failure	Replace pump seals.
Worn injection valve rotor	Replace rotor.
Clogged solvent reservoir filters	Clean or replace solvent reservoir filter.

If you have any questions regarding HPLC troubleshooting, contact our knowledgeable Technical Support Team toll free 1-800-267-8103 or by email at tech @chromspec.com.